Innate and Adaptive Immune Responses to SARS-CoV-2 in Humans: Relevance to Acquired Immunity and Vaccine Responses

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Introduction:

Since the advent of COVID-19 in Wuhan, China in December 2019, the virus has spread to virtually every country in the world now accounting for approximately 40 million cases world-wide with 1,113,434 deaths[1]. Despite these stark reminders of the morbidity associated with SARS-CoV-2 pneumonia, there remains large numbers of individuals who exhibit no or minimal symptoms despite demonstrating viral PCR positivity. Here, the factors responsible for the spectrum of COVID-19 disease severity and the genesis and nature of protective immunity against COVID-19 remain elusive. There are now multiple studies which have investigated the immune responses to COVID 19 in various populations, including those without evidence of COVID 19 infection[2-4]. These studies have yielded valuable information on human immune responses to COVID 19 and delivered insight into potential paths forward in discerning who would be at greatest risk form the virus based on immunologic assessments. In this paper, we will discuss emerging studies that examine innate and adaptive immune responses to SARS-CoV-2 and how they might be modified to protect individuals from collateral tissue injury induced by excessive innate immunity and induce long-lasting immunity to SARS-CoV-2 canonical antigens that are capable of eliciting long-lived T and B-cell immunity.

Despite efforts on many fronts, specific therapeutic approaches to treatment of SARS-CoV-2 have yielded minimal on no significant benefit compared to standard of care[5-9,36]. These include remdisivir, immune plasma, monoclonal antibodies against spike protein and anti-inflammatory agents. Currently, emerging vaccines hold the most hope for saving lives and stemming the epidemic. However, in the early days of the epidemic, the focus was on therapies aimed at controlling the cytokine storm that emerged in patients with SARS-CoV-2 pneumonia. Here, efforts were aimed at controlling elements of innate immunity that likely contribute to the morbidity and mortality of SARS-CoV-2 pneumonia. In this paper, we will discuss the relevant innate and adaptive immune responses developed by humans to SARS-CoV-2 and how they can be utilized to develop more rational therapeutic approaches to treatment of patients infected with COVID 19.

Innate Immune Responses to COVID 19: Interleukin 6

Early reports from patients with SARS-CoV-2 pneumonia identified interleukin 6 (IL-6) as a potential pathogenic factor in initiation of the acute respiratory distress syndrome (ARDS)[10]. IL-6 is a pleiotropic cytokine which functions as a mediator of

both innate and adaptive immune functions. IL-6 has diverse immune and biologic actions include direction of immune cell differentiation, sentinel responses to invading pathogens and ischemic injury. IL-6 is also critical for plasma cell growth, and immunoglobulin production. Excessive and unregulated IL-6 transcription is commonly seen in patients with autoimmune or inflammatory disorders[11]. Emerging data from patients with SARS-CoV-2 suggests IL-6 transcription is initiated and sustained after respiratory epithelium is infected. The virus had a proclivity for activation of alveolar and circulating macrophages resulting in copious and sustained IL-6 production resulting in the cytokine storm, endothelial cell damage, capillary leak and the clinical and pathological features of ARDS. This data suggests inhibiting IL-6 production and/or blocking receptor binding could be an important therapeutic option for limiting morbidity and mortality[10,12].

Here, tocilizumab (anti-interleukin-6 receptor [anti-IL-6R]) monoclonal is of interest due to its ability to reduce ARDS after chimeric antigen receptor T cell therapy (CART)cell therapy. Tocilizumab is a recombinant IgG1 humanized monoclonal antibody which inhibits the binding of IL-6 to the soluble and membrane-bound forms of the IL-6R. Tocilizumab is FDA approved for the treatment of severe rheumatoid arthritis, systemic juvenile idiopathic arthritis, giant cell arteritis, and more recently for cytokine release syndrome occurring after CART-cell therapy.

Our group and others have reported on the benefits of anti-IL-6R therapy for treatment of SARS-CoV-2 pneumonia[10,13-15]. However, press reports on two clinical trials of anti-IL-6R therapy, and a recently reported randomized clinical trial, failed to show benefit[16-18]. However, data from the EMPACTA trial showed that tocilizumab reduced the number of patients needing mechanical ventilation compared to placebo in a population of underserved and minority patients[19]. Also, very exciting and encouraging data released from the Remap-Cap international platform trial showed that tocilizumab significantly improved outcomes in the most severely ill patients with SARS-CoV-2 pneumonia[20]. Since tocilizumab was the first immune modulatory agent investigated in SARS-CoV-2 pneumonia, it has experienced many ups and downs in terms of results reported in real world experience and clinical trials that are often diametrically opposed. Certainly, it appears that not all patients would benefit from anti-IL-6R therapy, but emerging data suggest it is clear that blocking early innate immune responses to COVID 19 infection can be beneficial in severe SARS-CoV-2 pneumonia.

Innate Immune Responses to COVID 19: Complement

Little attention has been paid to potential role of complement activation in mediation of the severe manifestations of SARS-CoV-2 pneumonia. However, many symptoms could be attributed to systemic complement activation through the alternative, classic and possibly lectin binding pathways. These include ARDS and propensity to a hyper coagulable state. In this regard, there is likely interaction between elevated IL-6 levels seen in SARS-CoV-2 pneumonia patients and activation of the complement system. Here, IL-6 is a potent inducer of complement reactive protein (CRP)which has the ability to initiate complement activation. Recent reports have focused on evaluating the association of COVID 19 related inflammation with activation of the C5a-C5a receptor (C5aR) axis[21]. This paper examined the role of complement activation and specifically generation of the potent anaphylatoxin C5a in patients with COVID 19 infection. Patients were divided into four categories, healthy controls, COVID 19 patients with minimal symptoms, patients with pneumonia and finally those with severe ARDS. Blood levels of CRP, IL-6, C5a and chemokines associated with complement activation were examined. The investigators demonstrated a progressive and significant increase in all inflammatory markers evolving from minimal symptoms to ARDS. Importantly, they also examined lung samples from SARS-CoV-2 patients and found significant increase in macrophage and neutrophil infiltration with both cell types expressing high levels of C5a1 receptor. Broncho-alveolar lavage (BAL) fluid analysis showed increased levels of IL-6 and CXCL8 but C5a was detected in concentrations > 1000 pg/ml. The authors suggest that all 3 pathways to complement activation (classic, alternative and mannose binding lectin pathway [MBL/SP])are involved in SARS-CoV-2 induced pathology. In this regard, reports suggest that patients with the most intense anti-COVID antibody responses may develop more severe ARDS, likely due to classic pathway/alternative pathway complement activation by IgG/SARS-COV-2 immune complexes[22]. Importantly, these investigators evaluated how inhibition of the C5a/C5aR1 axis would effect markers of inflammation. Here in vitro experiments using a monoclonal antibody against C5aR1 showed with human cells showed that anti-C5aR1 inhibited C5a activation of neutrophils induced by high concentrations of C5a. Using a C5aR1 knock-in model of acute lung injury in mice, the investigators showed that anti-C5aR1 monoclonal markedly inhibited features of acute lung injury including neutrophil infiltration, IL-6 induction and albumin extravasation into alveoli. Pathologial feature were also

markedly improved with no evidence of ARDS in anti-C5aR1 treated animals. These observations suggest that modification of the C5a-C5aR1 axis could have benefit in treatment of patients with SARS-CoV-2 pneumonia[21].

Another important pathological consideration in patients with SARS-CoV-2 pneumonia is the proclivity for thrombotic events[23-25]. Here, intense complement activation is likely to cause activation of the coagulation system (with initiation of thrombotic events on the endothelium of blood vessels. Thus, inhibition of complement activation could prevent thrombotic complications of SARS-CoV-2 pneumonia.

Gao et al[26] have also suggested that the N protein of SARS-CoV-2 is a potent activator of the MBL/SP pathway and may be responsible for the the rapid development of ARDS in SARS-CoV-2 infected patients[27]. In this regard, C1 esterase inhibitor (C1-INH) regulates the intrinsic complement/coagulation pathway by inhibiting multiple pathways including Factor XII activation. Deficiency or loss of function of C1-INH would likely result in enhanced coagulation and fibrinolysis. This is supported by elevated blood D-dimer levels in patients with hereditary angioedema (HAE) resulting from C1-INH deficiency. Thus one could also surmise that use of C1-INH treatment could be of use in treating the manifestations of SARS-CoV-2 pneumonia, including inhibition of the innate immunity/coagulation pathway crosstalk. A recent report detailed the use of C1-INH treatment in 5 patients with SARS-CoV-2 pneumonia. Four of five showed rapid improvement in oxygenation, reductions in fever and CRP levels. They also showed a decline in complement activation products after treatment[28].

Data presented in the studies evaluated above suggest that investigation of complement inhibitors, especially those that can inhibit coagulation pathway activation hold promise in treatment of patients with SARS-CoV-2 pneumonia. In this regard, a recent paper by Vlaar et al[29] examined the utility of an anti-C5a monoclonal IFX-1 for treatment of patients with SARS-CoV-2 pneumonia. This was a small study which examined the PAO2/FiO2 ratios on day 5 after treatment compared to placebo. The study did not meet the primary endpoint, but of interest are the observations that pulmonary embolisms were reduced in anti-C5a treated patients (13% v. 40%) compared to placebo. There was also lower mortality at 28 days. However, caution must be taken since this is a small exploratory study not powered for those end points. Other trials of inhibitors of C3, C5, C5a, and C1INH are underway and should help elucidate whether or not complement

inhibition will have a role in treatment of patients with SARS-CoV-2 pneumonia[30].

Adaptive Immune Responses to COVID 19: B-cells & T-cells

Adaptive immunity involves the coordination of T and B-cell immune responses to the SARS CoV-2 virus. In this regard adaptive immunity is responsible for long-lasting and possibly sterilizing immunity to the virus. We now know that immune responses to the severe acute respiratory syndrome virus occurs within the first 7 to 10 days post infection. However, understanding the key features of this is still a conundrum. Is very important in the long run to ascertain the nature of the B-cell and T-cell immune events and whether they result in long-lasting immunity with memory B-cell/T-cell development or dissipate over time resulting in risk for recurrent infection and disease. These are also prescient issues for development of vaccines to combat the SARS-CoV-2 epidemic. In this section, we will focus on adaptive immune responses to SARS-CoV-2 and how to measure the strength and durability of the virus-specific immune responses.

Adaptive Immune Responses to COVID 19: Antibodies

With a rapid onset of the SARS-CoV-2 epidemic, critical information regarding immune responses to the virus have lagged as efforts focused on development of assays to detect antibody responses to the virus. We are now achieving a better understanding of the humoral immune responses to COVID-19. After the initial infection with COVID-19, early responses are IgM and IgA but is unclear if these can modify the course of the disease[2,31]. Subsequent IgG responses occur within 7 to 10 days post infection and would be expected to give sterilizing immunity to the virus, and with presumed development of memory B-cells, result in recall of high-affinity IgG anti-COVID-19 responses should re-exposure occur. However, it is known that the intensity, character and duration of IgG responses may vary greatly the IgG titers usually peak to peak at approximately 50 to 60 days post infection and may last up to 10 months[31-33]. It is also unknown if the disappearance of the antibody correlates with disappearance of specific memory to of the virus. There are now several cogent papers and that are beginning to address the nature and significance of IgG responses to the COVID-19 virus[32-35]. Is also known that intense immune responses to the virus of the IgG class is likely to cause severe cytokine release syndrome and may be associated with increased risk of death[21,22].

One of the cardinal features associated with an effective vaccine is developing neutralizing antibodies directed at spike protein. This is a basis for multiple clinical trials and also the basis for development of monoclonal antibodies cocktails that have been important in COVID-19 therapeutics short of vaccines. However, until recently little was known about what constitutes an effective

immune response to COVID 19. Here, an important consideration is the nature of antibodies aimed at the receptor binding domain (RBD) of SARS-CoV-2. In this regard recent papers have shown that antibodies binding to the receptor binding domain RBD are critical for long-term protective immunity to the COVID-19 infection and are associated with better patient survival[34,35]. These authors conclude that measuring antibodies to specific epitopes of SARS-CoV-2 antigens offers a more accurate assessment of sterilizing and clinically significant immunity. Recently Barnes et al[34] reported on how the structure and specificity of neutralizing antibody to SAR-CoV-2 inform therapeutic strategies. Using structural, biophysical and bioinformatics analyses of SARS-COV-2, the investigators analyzed approach angles of antibodies bound to RBDs on spike trimers. Their work provides a blueprint for designing antibody cocktails for therapeutics and potential COVID 19 spike-related immunogens for robust vaccine development. Thus, it is important to analyze the nature and specificity of the IgG responses to COVID-19. If antibodies are not directed at the RBD and cannot effectively bind spike trimers, they are likely to be ineffective in preventing infection. This should also be true of monoclonal antibody cocktails now being used for therapy in patients with SARS-CoV-2. Of interest in this regard is the use of convalescent plasma which has shown inconsistent or inconclusive results as a therapy[36]. This is likely due to variations in titer and avidity composition of IgG responses in patients recovering from SARS-CoV-2. In those which are directed at the receptor binding site if they bind in a way that prevents spike adherence to the ACE2 receptor are likely to prevent infections. In a recent report by Ibarrondo et al[37] the investigators examined the durability and robustness of anti-SARS-CoV-2 RBD directed antibodies in 34 patients with known or suspected infection with SARS-CoV-2. The investigators reported on an observed rapid decline in IgG antibodies directed at the SARS-CoV-2 RBD, indicating, in their opinion, that their observations "raise concern that humoral immunity against SARS-CoV-2 may not be long lasting in persons with mild illness, who compose the majority of persons with COVID-19". They also indicate that "the results call for caution regarding antibody-based "immunity passports," herd immunity, and perhaps vaccine durability, especially in light of short-lived immunity against common human coronaviruses". Given the information reported above, it is clear that a deeper understanding of the human immune response to COVID 19 is needed before such pronouncements can be made. First, early IgG responses emanate from germinal centers after T-follicular cells activate naïve B-cells to mature into activated B-cells that progress to B-memory cells and IgG producing plasmablast. Plasmablast are short-lived and with dissipation, the initial IgG responses are terminated. However, one should understand that this does not mean that immunity has waned. This is because of the persistence of B-memory cells and long-lived plasma

cells that reside in the bone marrow can reactivate antigen-specific responses to the SARS-CoV-2 RBD if re-exposed. In addition, this does not take into account the importance of T-cell memory for COVID-19 antigenic determinates that can result in direct cytotoxic T-cell immunity and help for B-cell responses[33]. Thus, the comments of Ibarrondo et al[37]need to be evaluated in the context of comprehensive immune responses to COVID-19 where redundancy, memory, diversity and durability are likely more important than initial IgG responses.

The monoclonal antibody cocktails have been developed so far seem to have some effect but there are no reports in the medical literature of efficacy. Initial reports from the Regeneron and Lilly on monoclonal IgG anti-spike protein cocktails suggests that they may prevent long-term symptoms in patients who are not hospitalized[38,40]. However, the impact of these antibodies on severe SARS-CoV-2 pneumonia appears minimal. Is also suspected that the larger antigenic burden is a major driver the magnitude of response to COVID-19 again this may be associated with intense immune responses with cytokine release syndrome. The monoclonal antibody cocktail developed by Lilly Pharmaceuticals has recently been discontinued for adverse events and lack of efficacy in hospitalized patients. However, recent emergency approval was given for outpatient use[39] . In addition, the Regneron monoclonal REGN-COV2 antibody cocktail recently showed it also improved symptoms in non-hospitalized patients[40]. The implementation of these therapies is likely to be difficult on a large scale due to the need for infusions in outpatient settings. These logistics will need to be resolved before wide implementation can be done. Also, the value and cost efficacy of these therapies in patients not sick enough to require hospitalization will need to be evaluated.

Adaptive Immune Responses to COVID-19: T-cells

With the rapidly evolving understanding of immune responses to the SARS-COv-2 virus, information on T-cell responses has taken center stage. In a series of interesting and extremely informative articles, we have gained much knowledge that is likely to change the way we look at viral-directed immune responses, the risk and severity of infection in individuals naïve to SARS-CoV-2 and the understanding of what constitutes an effective and sterilizing immune response. Importantly, it is critical to how we use this new information to improve the design of future vaccines.

One of the most interesting and provocative reports was by Braun et al[3]. These investigators examined CD4+ T-cell responses to the spike glycoprotein in the peripheral blood of patients with known SARS-CoV-2 infections as well as in healthy controls. Spike-reactive CD4+ T-cells were detected in 83% of infected individuals.

However, of greater interest, was the detection of spike-reactive CD4+ T-cells in the peripheral blood of 35% of healthy donors. It was noted that spike-reactive CD4+ T-cells in healthy donors were directed against C-terminal epitopes of the spike protein. The investigators also noted that spike-reactive T-cells against C-terminal epitopes have been identified in spike proteins of endemic coronaviruses which are responsible for seasonal upper respiratory tract infections. In unique and revealing experiments, the investigators showed that the SARS-CoV-2 reactive CD4+ T-cells from healthy donors also responded to the spike proteins of human endemic coronaviruses 229E and OC43. These findings suggest that the SARS-CoV-2 reactive T-cells found in healthy donors likely arose from previous exposure to the seasonal coronaviruses. The impact of this finding is unknown, however raise many important considerations. If one assumes that these CD4+ T-cells exert cross-reactive immune responses to SARS-CoV-2 infection, they may contribute to our understanding of the varying clinical phenotypes of COVID-19,and reported resilience of children and young adults to symptomatic SARS-CoV-2 infection. Here, children in day care centers where respiratory infections are common may have more frequent exposure to seasonal coronaviruses and chances to develop effective cross-reactive immunity. Other reports have also shown that SARS-CoV-2 infections are extremely rare in school age children. The investigators showed that after the reopening of primary schools in the UK, only 1 of 23,358 nasal swabs taken from children in June 2020 had detectable SARS-CoV-2, giving an estimate of 3.9 cases per 100,000 students. These authors give various reasons for this low infectivity rate but do not mention the possibility of activation of SARS-CoV-2 cross-reactive CD4+ T-cells as a possible factor in muting viral pathogenesis of SARS-CoV-2[41]. Although these inferences remain to be proven, further investigations into the breadth and vigor of SARS-CoV-2 responses in younger individuals could help in identifying those at lower risk for severe disease.

Further evidence for this hypothesis was presented by Mateus et al[4] who addressed the possible reasons for the reported detection of spike-protein cross-reactive T-cell memory in unexposed individuals. Here, using blood samples collected before SARS-CoV-2 was discovered (2015-2018) the investigators mapped 142 T-cell epitopes across the SARS-CoV-2 genome and demonstrated a range of pre-existing memory CD4+ T-cells with comparable affinity to those identified in patients recovering from SARS-CoV-2 infection. They also identified the likely source of these memory responses to cross-reactivity with coronaviruses responsible for the common cold. These authors also conclude that these pre-existing memory responses to SARS-CoV-2 are likely responsible for the variation in clinical phenotypes seen in patients with SARS-CoV-2 infection. In summary, the authors provide direct evidence that numerous CD4+ T

cells that respond to SARS-CoV-2 epitopes actually cross-react with corresponding homologous sequences from many different commonly circulating human coronaviruses and that these reactive cells are largely canonical memory CD4+ T cells. These findings of cross-reactive CD4+ T-cells specificities are in stark contrast to human coronavirus neutralizing antibodies, which are human coronavirus species specific and do not show cross-reactivity against SARS-CoV-2 receptor binding domains. These findings are quite remarkable and point to the primacy of CD4+ T-cells in creating effective and durable and cross-reactive immune responses to human coronaviruses, including SARS-CoV-2.

Zhang et al[42] examined the single cell profiles of immune cell responses to SARS-CoV-2 in patient with moderate and severe symptoms. The authors examined single-cell RNA sequencing in peripheral blood of 5 normal patients and 13 patients with SARS-CoV-2 pneumonia. The patients with SARS-CoV-2 had moderate or severe symptoms and some were convalescent cases. The authors looked at transcriptional profiles of T-cells and B-cells and also at determinants of the overall inflammatory response. Compared to normal individuals, most COVID-19 patients exhibited strong interferon-α (IFN-α) responses. The authors also identified a successful composition of CD4+ effector-GNLY (granulysin), CD8+ effector GNLY and NKT-CD160 (FcγR IIIb)+ cells that was associated with successful convalescence in moderate disease patients. However, in patients with severe disease, there were features of a deranged, excessive and persistent immune response. Here, persistent IFN-α responses resulted in T-cell exhaustion with skewed TCR repertoire and broad T cell expansion and the absence of NKT-CD160+ cell responses. The absence of NKT-CD160+ cells would suggest that the patients with more severe disease could not mediate viral elimination using antibody-dependent cell mediated cytotoxicity (ADCC) which may contribute to persistence of disease symptoms. This paper is important since it is the first to show how coordinated and focused immune responses to SARS-CoV2 are necessary for successful viral elimination and convalescence. The rapid expansion of IFN-α secreting cells in patients with SARS-CoV-2 pneumonia also suggests that the use of IFN-α therapy would not be advisable in the excessively inflamed individuals.

Peng et al[43] examined CD4+ and CD8+ T-cell memory responses using INF-γ responses to SARS-CoV-2 Spike peptides in 42 individuals who were recovering from SARS-CoV-2 infection. Fourteen with severe disease, 28 with mild disease and 16 unexposed individuals as controls. These investigators showed that T-cell responses were significantly higher in those with severe cases compared to milder cases. In this study, controls did not show any responses to COVID-19 spike peptides. T-cell responses also correlated with antibody production to spike peptides. These investigators also identified

41 peptides associated with SARS-CoV-2 that contained either CD4+ and/or CD8+ specific epitopes including six immunodominant regions that engendered responses in more than 50% of individuals. Of interest is the identification of CD8+ SARS-CoV-2 specific cells that were specifically identified as central and effector memory cells in patients with mild disease. Of critical importance is the identification of multiple strong and immunodominant responses of T-cells to non-spike (M and NP proteins) in 35% and 47% of patients respectively. They conclude that this finding may define established protective immunity and likely renders protection from serious infection with SARS-CoV-2. Since many of the immune responses were to non-spike proteins, this article highlights the importance of including immunodominant T-cell reactive non-spike peptides in future vaccine development.

Swadling and Maini[44] nicely summarized the findings of Peng et al[43] in an editorial titled *T-cells in COVID-19-united in diversity*. This succinct description of the complexities of the human immune response to SARS-CoV-2 and its relevance to identifying productive immune responses as well as implications for vaccine development are discussed. Functional CD4+ and CD8+ T-cell responses to multiple regions of SARS-CoV-2 were identified and appeared to be sustained. The authors bring up important points regarding the nature and durability of immunity to SARS-CoV-2. Certainly, those with more severe disease showed the most intense responses, but the presence of CD8+ central and effector memory cells with multiple epitopic specificities including to promiscuous non-spike proteins in those with milder disease likely cross-reactive with other coronaviruses. This appears to represent long-lasting and recognizant immunity. The authors also bring up the possibility that these CD8+ cells may reside in the respiratory tract to take on any new invasion of SARS-CoV-2 and could rapidly initiate responses after initial invasion. Even though the patients with the most intense responses are likely to retain them longer (i.e., neutralizing IgG antibodies and CD4+/CD8+ T-cells), it is still important consider that a small numbers of CD8+ memory cells can rapidly expand upon re-encounter with the virus and likely initiate effective immune responses. Unlike antibody which can result in a rapid sterilizing immunity, T-cells have to wait for antigen presentation and re-initiation of memory response before elimination of virus can be accomplished. Thus, this could explain the variable phenotype of disease presentation seen currently and the fact that asymptomatic individuals may carry virus until complete T-cell responses and antibody are generated.

In terms of understanding the duration and efficacy of T-cell responses to SARs-CoV-2, it is too early to determine this since long term studies will be needed in large populations. However, the data presented by Peng et al[43] are encouraging since T-cells

generated reacted with multiple epitopes on SARS-CoV-2. At this point, little information is available on the presence or duration of memory B-cells reactive to SARS-CoV-2. However, it is important to note that T-cell memory specific to SARS-CoV-1 could be detected 17 years after initial infection[45,46].

Understanding the Composition & Durability of Immunologic Memory to SARS-CoV-2

An interesting and informative paper recently published by Dan et al[33] explored the constituents of immunologic memory that developed after confirmed SARS-CoV-2 infection in 185 patients with 41 patients having more than one determination at approximately 6 months after initial infection. This study attempted to improve our understanding of the full complement of immunologic memory which has not yet been done. The authors simultaneously examined spike-specific IgG,IgA,IgM responses, spike-specific B-memory cells (B_m) and CD4+ and CD8+-T-cell responses specific for SARS-CoV-2. Patients studied exhibited the full range of clinical manifestations of SARS-CoV-2 infection. The value of this study is that it revealed real-world information on the kinetics humoral and cellular immune responses to COVID-19.

Spike-specific IgG responses (including IgG to RBD) were present in "almost all" individuals at 5 months post-COVID-19 infection. Due to lack of sampling frequency, the authors could not precisely determine the rate of decay of spike-specific IgG but found a broadly heterogenous initial spike-IgG response that did not configure into a stable or assessable memory profile. Thus, diversity in antibody responses to SARS-CoV-2 was the most consistent feature of humoral immune responses.

From my standpoint, the most interesting and novel aspect of this paper is the examination of B_m cell responses. The authors found that spike-specific B_m cells (CD19+,CD27+ IgD-) were found in "almost all" patients and did not demonstrate a determinable half-life. In fact, they appeared to increase up to 5 months post-SARS-CoV-2 infection. The importance of this observation cannot be overestimated. If confirmed, it could represent a long-lived recognizant B-cell and IgG response capacity. In fact, B_m responses have been detected up to 60 years after smallpox vaccination and greater than 90+ years after infection with the 1918 H1N1 influenza A virus[47,48].

To further understand the composition of B-cell immune responses, we have to return to the T-cell compartment. T-follicular-helper cells (T_{fh}) constitute a sub-set of CD4+ T-cells that are critical in activating naïve B-cell immune responses to antigens (SARS-CoV-2) in the germinal centers. Here, cytokines (IL-6 & IL-21) are

critical to drive naïve CD4+ T-cells to T_{fh} cells. Dan et al[33] examined circulating T_{fh} cells (cT_{fh}) specific for SARS-CoV-2 in their aforementioned patient population. Memory cT_{fh} cells were detected in 100% of patients infected with SARS-CoV-2. This memory appeared to be robust and persisted for more than 6 months.

CD4+ and CD8+ T-cell responses to SARS-CoV-2 peptides were also examined. The investigators found that most patients developed CD4+/CD8+ responses to SARS-CoV-2. Here, there was a slow decay observed over 6M. However, the investigators felt the responses were similar to those seen with Yellow Fever vaccines where the long-term durability could be ~10 years. This is similar to a recent report describing CD4+ T-cell responses to SARS-CoV-1, 17 years post-infection[45,46].

The authors offer several considerations of their work suggesting that there are few certainties regarding our understanding of how effective or how long our immune responses to SARS-CoV-2 or vaccines will last. However, there are reasonable assumptions that can be made. First, sterilizing immunity requires the presence of high-titer IgG anti-spike (RBD) antibodies. Short of this, it is not yet known how those with memory T & B-cell responses would handle subsequent encounters with the virus. Since memory B & T-cells have to be re-activated by antigen-presenting cells, they cannot deliver sterilizing immunity immediately. This process has to develop over time. Thus, an initial infection event with SARS-CoV-2 is needed, but is likely rapidly dissipated as immune activation events progress[44]. This would likely limit SARS-CoV-2 to a URI or "cold" like illness. Again, we cannot be sure of this and one must consider that analysis of cells from the peripheral blood likely does not represent resident SARS-CoV-2 reactive memory T & B-cells in lymphoid tissues of the upper respiratory tract and lungs which could result in more rapid and effective immunity. The author conclude that immune memory to SARS-CoV-2 consisting of at least three of five immunological compartments (IgG, B_m, CD4+,CD8+ T-cells) was measurable in ~90% of individuals more than 5 months after SARS-CoV-2 infection, indicating that durable immunity against COVID-19 disease is likely for most individuals. However, some individuals who exhibit poor memory responses may be susceptible to re-infection. More data is needed to completely understand the complexities and integration of immune responses to SARS-CoV-2.

Summary & Conclusions

Here, we have attempted to address the cogent issues regarding innate and adaptive immune responses to the novel SARS-CoV-2. As

noted, data regarding these issues are rapidly accumulating and helping the development of new therapeutic approaches for treatment of patients infected with SARS-CoV-2 and development of novel vaccines. At this writing, data of two novel vaccines has been released, both showing >95% efficacy in preventing SARS-CoV-2 infection[49,50]. This is so very important to all of us, especially to determine on a large scale if exposure to these vaccines can prevent disease. Here, it will be of interest to see data on the ability of these novel vaccines to initiate and sustain both antibody and T-cell mediated immune responses as was described above. Fortunately, we now have the tools to analyze both antibody and B & T-cell immune responses to SARS-CoV-2 antigens.

There was a surge in interest in analyzing and monitoring antibody responses as a measure of the presence and duration of immunity to SARS-CoV-2. However, data from several studies discussed here suggest that antibody can rapidly dissipate, lasting no more than 10 months. It is important to understand that this does not mean those infected have lost immunity to SARS-CoV-2 since T-cells and possibly memory B-cells have the capacity to recall and initiate sterilizing immune responses. We also discussed the nature of immune responses to SARS-CoV-2 and noted that investigators have identified productive immune responses that consist of granulysin producing T-cells and CD160+ NKT cells. + antibody. Investigators have also identified patients with severe infection who develop T-cell exhaustion and senescence, resulting in ongoing dysfunctional T-cell activation that may account for the manifestations of the post-viral inflammatory syndromes and possibly autoimmune manifestations seen in some patients[51]. This will be a new frontier for scientific investigation and analysis of post-SARS-CoV-2 infection related immune dysregulation.

Our review started with an examination of the innate immune responses which included cytokines and complement activation. The interventions directed at the IL-6/IL-6R JAK/STAT pathway have shown variable, but mostly disappointing results in treating patients with active SARS-CoV-2 pneumonia, however recent data are encouraging for use of tocilizumab in the most severely ill patients[20]. Despite demonstration of pathogenicity, anti-complement therapies have also not gained favor although clinical trials with anti-C5a are still underway[30]. Modification of innate immunity was the first attempt to modify the pathogenicity of SARS-CoV-2, but as noted above the focus has rapidly moved to a better understanding of adaptive immune responses and how this information can be used to design more effective and durable vaccines. Ultimately, this is the last best hope for controlling the pandemic and arming ourselves against future assaults from, as yet, unknown viral pathogens.

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